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Research Paper

Modification of Lipid-Protein Organisation of Thylakoid Membrane by Means of Cadmium Ion

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Abstract - *An elevated level of cadmium in the environment is known to interact with different parts of the living system there by causing serious environmental hazards. When isolated chloroplasts are incubated in presence of cadmium ion in an isotonic buffer medium, the metal ion (Cd^{2+}) was found to induce thylakoid membrane lipid peroxidation and damage of electron transport system. It retards the loss of pigments and degradation of proteins. The cation induced membrane lipid peroxidation causes a drastic modification of lipid-protein organization of chloroplasts as reflected from changes in absorption and emission characteristics of the organelle. The structural disorganization was attributed to the binding of metal ion to the thylakoid membrane surface through various functional groups viz. -SH, -COOH, -COOR, -OH etc. of proteins and lipids. Such a binding exposed the lipid-protein organization for an easier entry and attack of reactive oxygen species, mainly singlet oxygen. The retardation of pigment and protein loss was due to cation induced stacking of thylakoid membrane.*

Keywords: *Cadmium toxicity, chlorplasts, reactive oxygen species, thylakoid membrane, etc*.

Introduction

The stability of lipid-protein organization of thylakoid membrane in green plants plays a significant role in primary production (synthesis of carbohydrate) and environmental oxygenation [1].There are reports that the structural organization of thylakoid membrane is influenced by a number of exogenous factors like light, metal ions, hormone, pH of the suspension medium etc [2-5]. Particularly the cations cause drastic modification of thylakoid membrane which has been attributed to screening of surface negative charge and ion specific interaction with certain enzymes [3, 6]. The toxic elements are released to the environment from various natural and anthropogenic sources [7-10]. Due to their non-biodegradability and translocation within ecosystem, when their level exceed threshold limiting value these interact with different parts of living system thereby causing serious environmental hazards [11-14].Although detrimental effects of toxic elements have been extensively studied on animal systems, there are limited reports on the impact of toxic elements on plant system [15-17] Cadmium ion (Cd^{2+}) is known to affect the structure of thylakoid membrane through peroxidation and oxidative stress which subsequently leads to inactivation of oxygen evolving complex and dislocation of electron transport chain [17-18]. Cd^{2+} -ion substitutes Mn^{2+} -ion of oxygen evolving complex thereby inhibiting the reactions of PS – II [19] .But all these studies are limited to aging of chloroplasts *in vivo*.

In the present work an attempt has been made to study the impact of cadmium ion (Cd^{2+}), a well known toxic metal ion, on the lipid protein organization of photosynthetic membrane during the incubation of chloroplasts *in vitro*. The metal ion is found to cause drastic modification of thylakoid membrane system which is evident from cation induced enhancement of membrane lipid peroxidation, retardation in pigment and protein loss and changes in absorption and emission characteristics of chloroplasts.

Material and Methods

All the chemical were procured from local market and these were of analytical grade. Chloroplasts were isolated from primary leaves of 8 days old seeding of wheat (*Triticum aestivum* L.emend Thell CV Sonalika) grown on cotton soaked with distilled water at 25 $\pm$ 2^0 C under continuous illumination as described by Panda *et.al* 1986[3]. Pigments were extracted from isolated chloroplasts with prechilled 80% acetone and the extract was used for spectrophotometric determination of chlorophyll [20] and carotenoids[21] .The amount of MDA accumulated was determined as per the method described by Health and Packer [22]. 1ml of 0.5% TBA (Thiobarbituric Acid) in 20% TCA (Trichloroacetic Acid) was added to an equal aliquot of chloroplast solution along with few grains of acid washed sand (as boiling aid). The entire solution was heated on a water bath at 95^0C for 25 minutes and then centrifuged to clarify the solution. The absorbancy was measured at 532 nm and corrected for nonspecific turbidity by subtracting absorbancy at 600nm. The amount of MDA accumulated was estimated by using molar extinction coefficient, $155mM^{-1}$ cm^{-1} [22].

The concentration of protein was measured as per Lowry *et. al.* (1951) [23].The absorption spectra of isolated chloroplasts were scanned with a double beam UV-vis spectrophotometer (shimadzu – 1700, Japan) by double diffusion technique as per the method described by Thomas and Nagraja (1973)[24].

The room temperature fluorescence emission was measured by fluorescence spectrophotometer (Hitachi model 650-40, Japan) as per method described by Panda *et. al* 1987[25]. The chloroplasts solution equivalent to 5 µg/ml of chlorophyll in 50 mM Tris-HCl buffer containing 175mM of NaCl at pH 8.0 for different sets were excited at 450 nm for the measurement of fluorescence intensity at 685 nm (F_{685}).

Efficiency of electron transport was measured by DCPIP-Hill activity as per Biswal and Mohanty [26].

Results

The effects of cadmium ion (Cd^{2+}) on photosynthetic membrane lipid peroxidation during the incubation of chloroplasts in light and dark (measured through MDA estimation) are depicted in Fig.1. The rate of MDA accumulation is faster in light than in dark. The cation stimulates lipid peroxidation both in light and dark but the process is much faster in light than in dark. MDA accumulation increases sharply up to first few hours followed by a comparatively slow rise.

The impact of cadmium ion (Cd^{2+}) on total chlorophyll loss during the aging of isolated chloroplasts in light and dark are shown in Fig.2. The chlorophyll loss is much faster in light than in dark. The cation (Cd^{2+} -ion) retards the loss of chlorophyll both in light and dark.The cation (Cd^{2+} -ion) induced variation in total carotenoid loss during aging of isolated chloroplasts in light and dark are presented in Fig.3. The carotenoid loss is much faster in light than in dark. The cation retards the loss of carotenoids both in light and dark. It may be noted that the cation protects chloropylls more efficiently than carotenoids.

Fig.4 shows the effect of (Cd^{2+}) on the protein content of isolated chloroplasts incubated in light and dark. The cation retards protein degradation both in light and dark.

The cation (Cd^{2+}) induced changes in the position of the absorption peaks during the aging of isolated chloroplasts in light and dark are depicted in Table 1. The positions of absortion maxima at 678 nm (Red band) and 438 nm (Soret band or Blue Band) are shifted towards lower wavelength in presence of Cd^{2+}. The shift in absorption position is comparatively higher in light than in dark.

The effect of Cd^{2+} on the intensity of F_{685} during aging of cell free chloroplasts in light and dark is shown in Table 2. The cation enhances the loss of F_{685} both in light and dark but the loss was much higher in light than in dark.

The loss in the efficiency of DCPIP photoreduction by isolated chloroplasts in presence and absence of Cd^{2+} in light and dark is shown in Table 3. The rate of DCPIP-photoreduction declines at a very faster rate in light than in dark. Cd^{2+} further accelerates the loss of DCPIP-Hill activity.

Discussion

From Fig.1, it is clear that thylakoid membrane lipid peroxidation occurs at a very faster rate up to first few hours followed by a slow rise both in presence and absence of cadmium ion. This may be attributed to photoinduced generation of reactive oxygen species (mainly $O_2^{\bullet-}$) through electron transport chain and excitation transfer from triplet chlorophyll to triplet oxygen generating singlet oxygen and other reactive oxygen species like hydroxyl radical, hydrogen peroxide etc [25, 27,28].The electron transport chain collapses after first few hours (Table 3). A significant enhancement of peroxidation even after the complete loss of electron transport chain suggests that chlorophylls which are still present in significant amount may induce the process through generation of singlet oxygen and other reactive oxygen species like hydroxyl radical, hydrogen peroxide etc. The lipid perioxidation in dark, may be due to the presence of residual reactive oxygen species generated during the isolation of chloroplasts from light grown seedlings [3-6, 25]. The Cd^{2+} ion induced enhancement of membrane lipid peroxidation may be attributed to direct binding of metal ion to the membrane surface through –SH, -COOH, -OH groups of proteins and –COOH, -COOR groups of lipids [6,29]. Such types of linkages expose unsaturated lipids of thylakoid membrane to different reactive oxygen species for peroxidation. The direct binding of metal ion to thylakoid membrane surface is evident from a blue shift of absorption peaks (Table 1) and lowering of chlorlophyll–a fluorescence intensity (Table 2). It is quite interesting to note that although the cation is enhancing membrane lipid peroxidation, it

is retarding the loss of pigments and proteins (Fig. 2, 3 and 4). This may be explained in terms of cation induced aggregation of chlorophylls and chlorophyllides which protects them from further degradation. Secondly the cation induced retardation of chlorophyll loss was higher than that of carotenoid loss. Since carotenoids are well known quenchers of singlet oxygen [27, 30], their faster degradation may induce the formation of larger amount of singlet oxygen resulting in peroxidation of membrane lipids. Since singlet chlorophylls [^{1}(Chl)*] mostly participate in primary reactions of chloroplasts [31,32], the cation induced damage of photochemical potential (Table 3) may reflect accumulation of triplet chlorophyll [^{3}(Chl)*]. This fact is well supported by quenching of chlorophyll-fluorescence (Table 2). The cation induced retardation in protein loss may be attributed to specific binding of cation with protein moiety thereby preventing from degrative forces.

Conclusion

From the above experimental facts and discussion, it is clear that Cd^{2+} causes degradation of thylakoid membrane (shown from Lipid peroxidation) and also induces its stacking (shown from pigment and protein degradation). But the degradation predominates stacking as evident from blue shift of absorption peak, lowering of Chl-a fluorescence intensity and damage of electron transport system. Thus, cadmium ion is highly toxic to the photosynthetic organelle.

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Table 1: Effect of Cd^{2+} position of absorption peak of chloroplasts incubateld in light and dark. R →Red peak at 678 nm, B→ Blue peak at 438 nm.

Time (Hr)	Absorption Peak Position (nm)							
	Control				Cd^{2+} ion treated			
	Light		Dark		Light		Dark	
	B	R	B	R	B	R	B	R
0	438	678	438	678	438	678	438	678
12	435	675	438	677	434	673	436	676

Table 2: Effect of Cd^{2+} chlorophyll-a fluorescence intensity (F_{685}) of chloroplasts incubated in light and dark.

Time (Hr)	Fluorescence intensity at 685 nm			
	Control		Cd^{2+} ion treated	
	Light	Dark	Light	Dark
0	100	100	100	100
6	42.8	67.0	36.5	53.0

Table 3: Changes in the rate of DCPIP-Hill reaction of chloroplasts incubated in presence and absence of Cd^{2+} light and dark.

Time (Hr)	Percentage of DCPIP-Hill activity			
	Control		Cd^{2+} ion treated	
	Light	Dark	Light	Dark
0	100	100	100	100
1	40	70	27	41
2	20	52	05	21
3	08	32	0	05
4	0	12	0	0

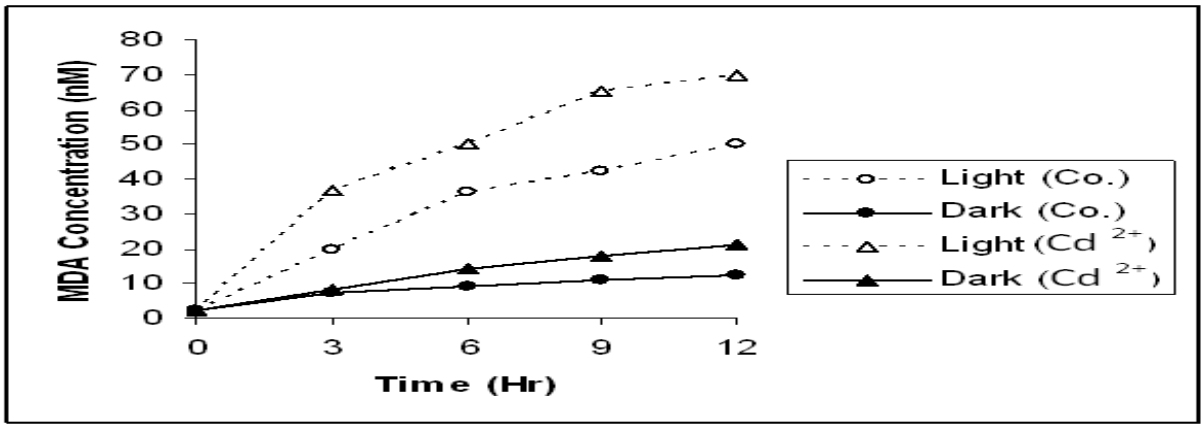

Fig.1. Effect of Cd^{2+} MDA concentration during aging of chloroplasts *in vitro* (Light and Dark)

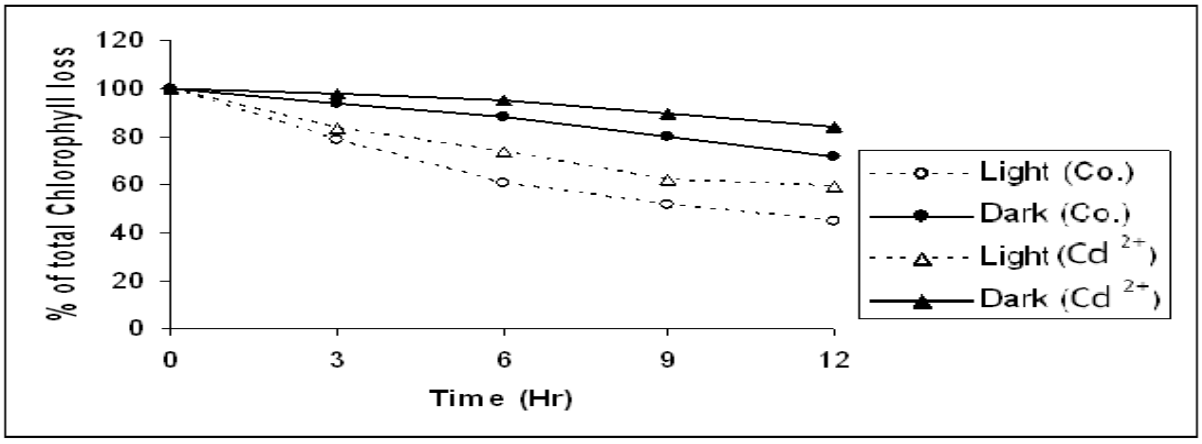

Fig. 2. Effect of Cd^{2+} total chlorophyll loss during aging of chloroplasts in light and dark.

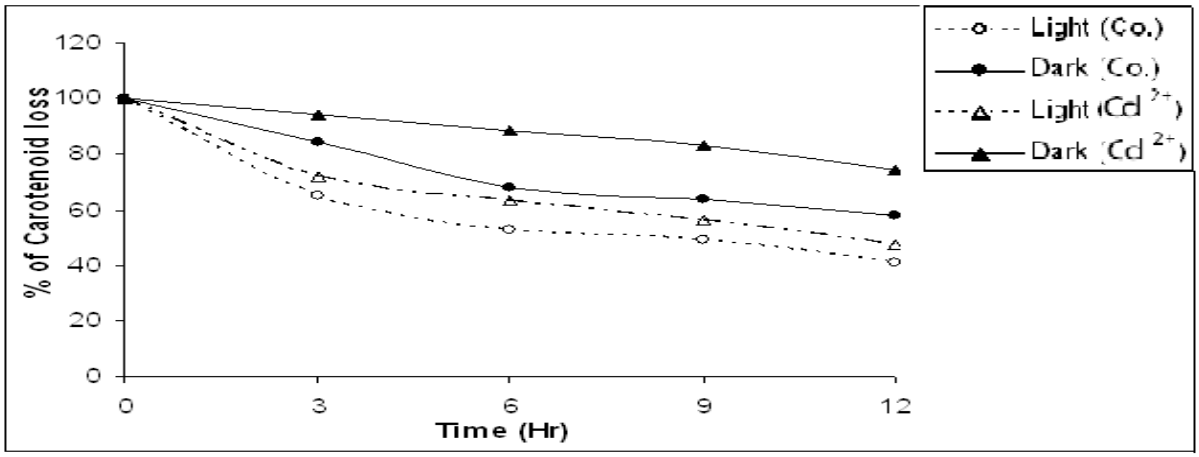

Fig. 3. Effect of Cd^{2+} ion on caroteniod loss during aging of chloroplasts in Light and Dark.